Quantification of root-soil and root-insect interactions using X-ray microtomography

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Introduction

X-ray microtomography is rapidly developing as a technique to investigate both the structure of soils and rhizosphere interactions (Gregory *et al*., 2003). The technique relies on biological tissue (e.g. roots) attenuating X-rays less than the surrounding soil. X-rays are passed through a soil microcosm containing a living plant and/or root-feeding insects and detected at the other side (Fig. 1). Each cubic unit (or voxel) within the soil microcosm is assigned an attenuation value based on the extent to which the X-rays have been attenuated.

This information is then used to reconstruct an image of the contents of the microcosm. Specialist benchtop instruments have been developed that allow spatial resolution of better than 100µm for sample sizes of up to 50 mm diameter. This poster reports studies on the growth of wheat roots in response to a band of phosphate and on the movement of insects (*Sitona lepidus* and *Otiorhynchus sulcatus*) in the rhizosphere.

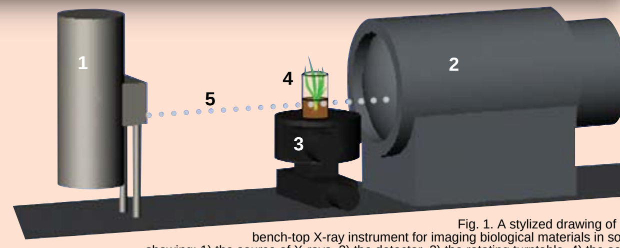

Fig. 1. A stylized drawing of a bench-top X-ray instrument for imaging biological materials in soil showing: 1) the source of X-rays, 2) the detector, 3) the rotating turntable, 4) the soil microcosm and plant/insect, and 5) the path of the X-ray beam

Study 1

Pre-germinated wheat (*Triticum aestivum* L.) seeds were grown in pots of 210 mm diameter acrylic tubing, 250 mm long, lined with a polythene bag. The pots were filled in three layers: the upper and lower layers consisted of acid washed sand (air-dried, sieved to <2 mm), pre-wet to a gravimetric water content of 17.5% with de-ionised water. The middle layer consisted of Bullionfield soil (a sandy loam, air-dried and sieved to <2 mm), also pre-wet to a gravimetric water content of 17.5%, but with nutrient solution (0.5 g l^{-1} KH_2PO_4). The soil layer was 2 cm deep and the upper sand layer 3cm deep. The main root axes contacted the soil band within two days of germination. Lateral roots were visible 4 days later, and 8 days after germination, an extensive array of lateral roots was visible on the radicle within the soil band (Fig.2).

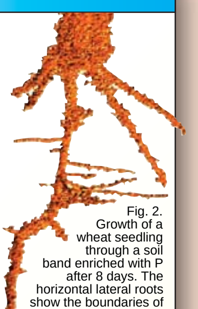

Fig. 2. Growth of a wheat seedling through a soil band enriched with P after 8 days. The horizontal lateral roots show the boundaries of the soil band.

Study 2

The movement of *Sitona lepidus* larvae towards nodules of white clover (*Trifolium repens* L.) roots growing in re-packed soil (Sonning series [sandy loam] sieved to <75 µm) was studied in columns 20mm diameter and 30 mm high (Johnson *et al*., 2004). A single neonatal larva (< 1 mm long) was introduced to each column and its position measured by X-ray tomography at 0, 3, 6 and 9h (Fig. 3).

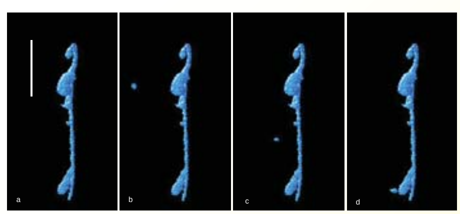

Fig. 3. Sequential images showing the movement of a neonatal *Sitona lepidus* larva towards the nodule of a white clover root. The white bar is 10 mm.

The larvae traveled between 9 and 27 mm in 9h at a mean speed of 1.8 mm h^{-1}. Burrowing patterns were convoluted rather than linear with changes in trajectory evident from this study that would be masked in more commonly used "slant boards". These studies demonstrated that the larvae responded to a chemical signal from the nodule/root, possibly an isoflavonoid (Johnson *et al*., 2005).

Study 3

Most recently, studies using a bench-top scanner (X-TEK Group, Herts, U.K., (http://www.xtekxray.com/systems.htm#bt) with a 5 µm focal spot reflection target and an X-ray source with an operating regime of 25-160kV have allowed reconstruction of both biological materials and soil structure. Fig. 4 shows a neonatal black vine weevil, *Otiorhynchus sulcatus*, larva (~ 1 mm body length) surrounded by soil particles. These data were acquired at 90kV, 125 µA using a tungsten target and 720 angular positions. Reconstruction of the data, in Hounsfield units, was obtained for an isotropic voxel size of 0.029038 mm using a filtered back-projection algorithm.

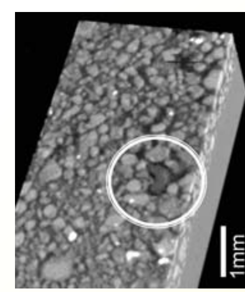

Fig. 4. A neonatal larva of the black vine weevil (*O. sulcatus*) and the surrounding soil particles.

Conclusions

- Further improvements in the spatial resolution of bench-top X-ray tomographic instruments (currently approaching 5 µm for samples 50 mm in diameter) will allow finer roots and smaller soil organisms to be imaged.
- Such systems will allow many aspects of the "black box" of belowground systems to be resolved.

References

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Acknowledgement

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